

IN THE CLAIMS:

Please amend claims 1, 6, 9, 10, 21, 23, and 37 as follows.

1. (Amended) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

wherein X is a pharmacologically active peptide sequence, and

wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R² together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring, e.g. 2,4-diaminobutanoic acid and 2,3-diaminopropanoic acid; and

wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2; or a salt thereof.

C² 6. (Amended) A peptide conjugate according to claim 1, wherein Z consists of about 4 to about 15 amino acid units.

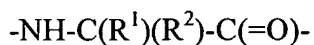
9. (Amended) A peptide conjugate according to claim 1, wherein Z comprises at least one Lys amino acid unit.

C³ 10. (Amended) A peptide conjugate according to claim 9, wherein Z is (Lys)_n, wherein n is an integer in the range from about 4 to about 15.

C⁴ 21. (Amended) A peptide conjugate according to claim 1, wherein Z is (Dbu)_n or (Dpr)_n, wherein n is an integer in the range from about 4 to about 15.

C⁵ 23. (Amended) A peptide conjugate according to any one of the preceding claims, wherein said pharmacologically active peptide sequence (X) consists of at the most 75 amino acid units.

C⁶ 37. (Amended) A method for inhibiting neurons from transmitting pain impulses to the spinal cord, comprising administering to a subject in need thereof a conjugate comprising enkephalin and a stabilising sequence Z of 4-20 amino acids covalently attached by its N terminus to the C terminus end of X, wherein Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



(I)

wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R²

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together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring;

wherein the ratio between the half-life of said peptide conjugate and the half-life of enkephalin when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2, said peptide conjugate being orally absorbed in an amount effective to inhibit neurons from transmitting pain impulses.

Please cancel claims 39, 41, 43, 45, 47 and 49 without prejudice.

Kindly add the following new claims 52-⁶⁸~~67~~.

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52. (New) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

wherein X is a pharmacologically active peptide sequence and

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wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X, wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R²

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together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring; and

wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 3; or a salt thereof.

53. (New) The peptide conjugate of claim 52, wherein the ratio is at least about 5, 7, 9, or 10.

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54. (New) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

wherein X is a pharmacologically active peptide sequence, and

wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X, wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



wherein R^1 and R^2 are selected from the group consisting of hydrogen, C_{1-6} -alkyl, phenyl, and phenyl-methyl, wherein C_{1-6} -alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C_{1-6} -alkyl, C_{2-6} -alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R^1 and R^2 together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring; and

Sub D5 und } wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2; or a salt thereof, wherein Z consists of about 4 to about 10 amino acids.

DEFI } 55. (New) The peptide conjugate of claim 54, wherein Z consists of about 4 to about 7 amino acid units.

56. (New) The peptide conjugate of claim 55, wherein Z consists of 6 amino acid units.

C 7 } 57. (New) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

wherein X is a pharmacologically active peptide sequence, and

Sub D6 } wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X, wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R²

together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring; and

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wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2; or a salt thereof, wherein Z comprises at least two or three Lys amino acid units.

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58. (New) The peptide conjugate of claim 57, wherein Z comprises at least four or five Lys amino acid units.

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59. (New) The peptide conjugate of claim 58, wherein Z comprises six Lys amino acid units.

60. (New) The peptide conjugate according to claim 9 or 57, wherein Z is (Lys)_n, wherein n is an integer in the range from about 4 to 10.

61. (New) The peptide conjugate of claim 9 or 57, wherein Z is (Lys)_n, wherein n is an integer in the range from about 4 to 8 or about 4 to 6.

62. (New) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

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wherein X is a pharmacologically active peptide sequence, and

wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X, wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



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wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R² together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring; and

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wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2; or a salt thereof, wherein Z is (Dbu)_n or (Dpr)_n, wherein n is an integer in the range from about 4 to about 10.

63. (New) The peptide conjugate of claim 62, wherein Z is (Dbu)_n or (Dpr)_n, wherein n is an integer such as in the range from 4 to 6.

64. (New) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

wherein X is a pharmacologically active peptide sequence, and

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wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X, wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



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wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R² together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring; and

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wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2; or a salt thereof, wherein said pharmacologically active peptide sequence (X) consists of at the most about 65 amino acid units.

65. (New) The peptide conjugate of claim 64, wherein said pharmacologically active peptide sequence (X) consists of at the most about 60, about 55, about 53 or about 50 amino acid units.

66. (New) A method for inhibiting neurons from transmitting pain impulses to the spinal cord, comprising administering to a subject in need thereof a conjugate comprising enkephalin and a stabilising sequence, Z of 4-20 amino acids covalently attached to X, wherein Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



wherein R¹ and R² are selected from the group consisting of hydrogen, C₁₋₆-alkyl, phenyl, and phenyl-methyl, wherein C₁₋₆-alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C₁₋₆-alkyl, C₂₋₆-alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfono, and carboxy, or R¹ and R² together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring, e.g. 2,4-diaminobutanoic acid and 2,3-diaminopropanoic acid;

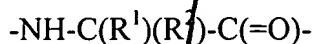
wherein the ratio between the half-life of said peptide conjugate and the half-life of enkephalin when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 3, said peptide conjugate being orally absorbed in an amount effective to inhibit neurons from transmitting pain impulses.

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67. (New) The method of claim 66, wherein the ratio is at least about 5, about 7, about 9 or about 10.

68. (New) A pharmacologically active peptide conjugate having a reduced tendency towards enzymatic cleavage comprising X and Z,

Sub D₉ } wherein X is a pharmacologically active peptide sequence, and

wherein Z is a stabilising peptide sequence of 4-20 amino acid units covalently bound by its N terminus to the C terminus end of X, wherein each amino acid unit in said stabilising peptide sequence Z is selected from the group consisting of Ala, Leu, Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Lys, Arg, His, Met, Orn, and amino acid units of the general formula I



(I)

wherein R^1 and R^2 are selected from the group consisting of hydrogen, C_{1-6} -alkyl, phenyl, and phenyl-methyl, wherein C_{1-6} -alkyl is optionally substituted with from one to three substituents selected from halogen, hydroxy, amino, cyano, nitro, sulfonyl, and carboxy, and phenyl and phenyl-methyl is optionally substituted with from one to three substituents selected from C_{1-6} -alkyl, C_{2-6} -alkenyl, halogen, hydroxy, amino, cyano, nitro, sulfonyl, and carboxy, or R^1 and R^2 together with the carbon atom to which they are bound form a cyclopentyl, cyclohexyl, or cycloheptyl ring, e.g. 2,4-diaminobutanoic acid and 2,3-diaminopropanoic acid; and

wherein the ratio between the half-life of said peptide conjugate and the half-life of the corresponding pharmacologically active peptide sequence X, when treated with carboxypeptidase A or leucine aminopeptidase in about 50 mM phosphate buffer solution at about pH 7.4 at about 37°C or in serum or plasma is at least about 2; or a salt thereof, wherein,

Z is Lys_p-Xaa_q or Xaa_p-Lys_q , wherein p and q are integers in the range from 1 to 14, with the proviso that $p+q$ is in the range of 3-15, and each Xaa is independently selected from the group consisting of Ser, Thr, Tyr, Asn, Gln, Asp, Glu, Arg, His, Orn, 2,4-diaminobutanoic acid, 2,3-diaminopropanoic acid and Met, and further wherein,

X is selected from the group consisting of AF 12505 (Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala) (SEQ ID NO. 14), insulin-like growth factor I (57-70) (Ala-Leu-Leu-Glu-Thr-Tyr-Cys-Ala-Thr-Pro-Ala-Lys-Ser-Glu) (SEQ ID NO. 15), insulin-like growth factor I (30-41) (Gly-Tyr-Gly-Ser-Ser-Ser-Arg-Arg-Ala-Pro-Gln-Thr) (SEQ ID NO. 16), insulin-like growth factor I (24-41) (Tyr-Phe-Asn-Lys-Pro-Thr-Gly-Tyr-Gly-Ser-Ser-Ser-Arg-Arg-Ala-Pro-Gln-Thr) (SEQ ID NO. 17), insulin-like growth factor II (33-40) (Ser-Arg-Val-Ser-Arg-Arg-Ser-Arg) (SEQ ID NO. 18), insulin-like growth factor II (33-40) (Tyr-Ser-Arg-Val-Ser-Arg-Arg-Ser-Arg) (SEQ ID NO. 19), insulin-like growth factor II (69-84) (Asp-Val-Ser-Thr-Pro-Pro-Thr-Val-Leu-Pro-Asp-Asn-Phe-Pro-Arg-Tyr) (SEQ ID NO. 20), growth hormone (GH)-releasing peptide-6 (GHRP-6) (His-DTrp-Ala-Trp-DPhe-Lys-NH₂) (SEQ ID NO. 21), beta-Interleukin I (163-171) (Val-Gln-Gly-Glu-Glu-Ser-Asn-Asp-Lys) (SEQ ID NO. 22), beta-Interleukin II (44-56) (Ile-Leu-Asn-Gly-Ile-Asn-Asn-Tyr-Lys-Asn-Pro-Lys-Leu) (SEQ ID NO.

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23), Interleukin II (60-70) (Leu-Thr-Phe-Lys-Phe-Tyr-Met-Pro-Lys-Lys-Ala) (SEQ ID NO. 24),
exendin-4 (GLP-1 analog) (His-Gly-Glu-Gly-Thr-Phe-Thr-Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-
Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-
Pro-Pro-Ser-NH₂) (SEQ ID NO. 25), exendin-3 (GLP-1 analog) (His-Ser-Asp-Gly-Thr-Phe-Thr-
Ser-Asp-Leu-Ser-Lys-Gln-Met-Glu-Glu-Glu-Ala-Val-Arg-Leu-Phe-Ile-Glu-Trp-Leu-Lys-Asn-
Gly-Gly-Pro-Ser-Ser-Gly-Ala-Pro-Pro-Pro-Ser) (SEQ ID NO. 26), epidermal growth factor (20-
31) Cys(Acm)-Met-His-Ile-Glu-Ser-Leu-Asp-Ser-Tyr-Thr-Cys(Acm) (SEQ ID NO. 27),
bivalirudin (Hirulog) (D-Phe-Pro-Arg-Pro-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-
Tyr-Leu) (SEQ ID NO. 28), hirulog-1 D-Phe-Pro-Arg-Pro-(Gly)₄-Asn-Gly-Asp-Phe-Glu-Glu-
Ile-Pro-Glu-Tyr-Leu (SEQ ID NO. 29), C-type natriuretic peptide (1-53) (CNP) (Asp-Leu-Arg-
Val-Asp-Thr-Lys-Ser-Arg-Ala-Ala-Trp-Ala-Arg-Leu-Leu-Gln-Glu-His-Pro-Asn-Ala-Arg-Lys-
Tyr-Lys-Gly-Ala-Asn-Lys-Lys-Gly-Leu-Ser-Lys-Gly-Cys-Phe-Gly-Leu-Lys-Leu-Asp-Arg-Ile-
Gly-Ser-Met-Ser-Gly-Leu-Gly-Cys; Disulfide bridge: Cys37-Cys53) (SEQ ID NO. 30), "Mini
ANP" (Met-Cys-His-cyclohexylAla-Gly-Gly-Arg-Met-Asp-Arg-Ile-Ser-Cys-Tyr-Arg, disulfide
bridge cys2-cys13) (SEQ ID NO. 31), Melanotan-II (MT-II, alpha-MSH4-10-NH₂, or Ac-Nle4-
Asp5-His6-D-Phe7-Arg8-Trp9-Lys10) (SEQ ID NO. 32), thymosin alpha1 (TA1) (Ac-Ser-Asp-
Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-
Glu-Glu-Ala-Glu-Asn) (SEQ ID NO. 33), Cys-Phe-Ile-Gln-Asn-Cys-Pro-Orn-Gly-NH₂,
Disulfide bridge: Cys1-Cys6) (SEQ ID NO. 34), octreotide (201-995) (DPhe-Cys-Phe-DTrp-
Lys-Thr-Cys-Thr-ol; disulfide bridge: Cys2-Cys7) (SEQ ID NO. 35), calcitonin gene-related
peptide (CGRP) (Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-
Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH₂,
Disulfide bridge: Cys2-Cys7) (SEQ ID NO. 36), endomorphin-1 Tyr-Pro-Trp-Phe-NH₂ (SEQ ID
NO. 37); endomorphin-2 Tyr-Pro-Phe-Phe-NH₂ (SEQ ID NO. 38), nociceptin (also known as
Orphanin FQ, Phe-Gly-Gly-Phe-Thr-Gly-Ala-Arg-Lys-Ser-Ala-Arg-Lys-Leu-Ala-Asn-Gln)
(SEQ ID NO. 39), angiotensinogen (1-13) (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-
His) (SEQ ID NO. 40), adrenomodullin (1-12) (Tyr-Arg-Gln-Ser-Met-Asn-Asn-Phe-Gln-Gly-
Leu-Arg) (SEQ ID NO. 41), antiarrhythmic peptide (AAP) (Gly-Pro-Hyp-Gly-Ala-Gly) (SEQ ID
NO. 42), Antagonist G (Arg-DTrp-(nMe)Phe-DTrp-Leu-Met-NH₂), indolicidin (Ile-Leu-Pro-
Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-NH₂) (SEQ ID NO. 43), osteocalcin (37-49) (Gly-

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